4. MUTAGENICITY

4.1. INTRODUCTION

The mutagenic effects of 1,3-butadiene have been reviewed extensively (Rosenthal, 1985; de Meester, 1988; Arce et al., 1990; Norppa and Sorsa, 1993; Jacobson-Kram and Rosenthal, 1995). The last of these reviewed publications through 1994 are on the genetic effects associated with butadiene (and metabolites). There is extensive evidence that butadiene and the two primary epoxide metabolites (epoxybutene and diepoxybutane) induce genotoxic effects in a variety of in vitro and in vivo test systems. Most of the in vivo studies discussed in the cited reviews were assays in mice and rats using cytogenetic endpoints, and the results generally support the dichotomy in carcinogenic response where mice are more responsive than rats. This review will focus on recently published studies performed in vivo (both somatic and germ cell effects) with an emphasis on those studies providing information relative to the mode of action of butadiene metabolites.

4.2. GENE MUTATIONS

Most of the earlier in vivo genotoxicity studies used cytogenetic endpoints (aberrations, micronuclei, or sister chromatid exchange [SCE]). It is recognized that this reflected the dearth of in vivo assays measuring gene mutations and limited the interpretation of in vitro versus in vivo findings. The ability to detect mutations at the *hprt* locus obtained from T lymphocytes from exposed mammals including mice, rats, monkeys, and humans provides an important step in developing an understanding of chemically induced mutational processes. Cochrane and Skopek (1993, 1994a) used $B6C3F_1$ mice and human TK6 cells to evaluate the mutagenic potential of butadiene and the two major metabolites. In the in vivo studies, mice were exposed for 6 h/day, 5 days/week for 2 weeks to butadiene at 625 ppm. The induced *hprt* mutant frequency was 6.2×10^{-6} compared with 1.2×10^{-6} from unexposed controls. For the metabolites, mice received three daily intraperitoneal (i.p.) injections of 60, 80, or 100 mg/kg of epoxybutene or 7, 14, or 21 mg/kg of diepoxybutane. Mutant frequencies in *hprt* from splenic T cells were dose related for both metabolites, with maximal responses of 8.6×10^{-6} and 13×10^{-6} for epoxybutene and diepoxybutane, respectively. Similarly, they found diepoxybutane about 100 times more effective than epoxybutene when human lymphoblastoid TK6 cells were treated in vitro.

In a recent meeting presentation, Meng et al. (1996) reported on a study in which both mice and rats were exposed by inhalation to 1,250 ppm butadiene for 2 weeks (6 h/day, 5 days/week). Groups of animals were necropsied before exposure (controls) and weekly up to 10 weeks after the last exposure. The researchers measured *hprt* mutants in both spleen and thymus

using the T-cell cloning assay. Mutant frequencies in both tissues of both species increased for several weeks and then declined. Maximal frequencies were: in thymus, 1.3×10^{-6} in mice (2 weeks) and 4.9×10^{-6} in rats (3 weeks); in spleen, 19.7×10^{-6} in mice (5 weeks) and 8.4×10^{-6} in rats (4 weeks). They determined a relative mutagenic potency (RMP) as the ratio of cumulative increase in mutant frequency in treated versus controls. For the spleen the RMP was 7.18 for mice compared with 2.04 for rats.

Several recent studies have measured in vivo mutations using the phage *lacI* or *lacZ* genes incorporated into a rodent genome. Recio and Goldsworthy (1995) summarized several experiments in which male B6C3F$_1$ *lacI* transgenic mice were exposed to 62.5, 625, and 1,250 ppm butadiene (6 h/day, 5 days/week) for 4 weeks. Two weeks after the last exposure, animals were euthanized and DNA was extracted from bone marrow to be examined for *lacI* mutagenesis. Mutations increased in a dose-response manner, reaching an apparent plateau at 625 ppm (about a fourfold increase above controls). Sequence analysis of *lacI* mutant colonies from the 625 and 1,250 ppm groups indicated an increased frequency of point mutations at A:T base pairs. These findings are consistent with those observed in butadiene-induced *hprt* mutant T lymphocytes from B6C3F$_1$ mice (Cochrane and Skopek, 1994b).

Several studies of genetic effects in exposed workers have recently been reported. Ward et al. (1994, 1996b) measured the frequency of *hprt* mutations in lymphocytes of workers in a butadiene production plant (two studies) and in a styrene-butadiene rubber plant. In the first study exposure estimates were based on 8 h samples in two production areas and in a central control area. Mean butadiene concentration in the production areas was 3.5 ppm, but the majority of samples showed concentrations below 1 ppm; mean butadiene concentration in the control was 0.03 ppm. Variant frequencies at the *hprt* locus in PHA-stimulated peripheral blood T cells of a high exposure group were increased more than threefold compared with the low-exposure and nonexposed groups. The eight individuals in the high-exposure group had *hprt* variant frequencies varying from 0.94×10^{-6} to 8.98×10^{-6} and the variant frequency generally correlated with the level of the metabolite dihydroxybutane in the urine. Whether the difference was due to differences in exposure or genetic differences in metabolism cannot be ascertained from the data. A second study was conducted in the same plant about 1 year later (Ward et al., 1996b). Measured butadiene concentrations in personal samplers were markedly lower, 0.30 ± 0.59, 0.21 ± 0.21, and 0.12 ± 0.27 ppm in areas defined as high, medium, and low exposure (no controls were reported for the second study). The corresponding *hprt* variant frequencies were 5.33 ± 3.76, 2.27 ± 0.99, and $2.14 \pm 0.97 \times 10^{-6}$, respectively. Individual data were not reported for this study, but again there is a high standard deviation in the highly exposed group. The Ward et al. (1996b) paper also reported preliminary results from workers in a styrene-butadiene rubber plant. Workers were assigned to high (20 of 40 personal samplers exceeded the 0.25 ppm

detection limit and 11 had a concentration over 1 ppm) and low (none of 26 exceeded the detection limit) exposure groups. In nonsmokers, the *hprt* variant frequencies were 7.47 ± 5.69 and 1.68 ± 0.85 × 10^{-6} for the high and low groups, respectively. While the variant frequency for smokers in the high-exposure group (6.24 ± 4.37) was not different from nonsmokers, the frequency for smokers in the low exposure group was about twice the nonsmoker group (3.42 ± 1.57). These preliminary findings with small sample sizes and no detail about smoking history or other confounding factors raise several unanswerable questions. The autoradiographic procedure for detecting *hprt* variants was used in these studies. The limitation of this method is that it is not possible to distinguish between several independent mutations and a single mutation giving rise to a clone of cells with the mutant phenotype. The procedure using the T lymphocyte cloning assay and subsequent DNA sequence analysis of clones as described by Albertini et al. (1982), and Recio et al. (1990) provide sufficient data for ascertaining independent mutational events.

Hayes et al. (1996) employed the T cell cloning assay to detect mutant frequencies in lymphocytes of workers in a rubber production factory. Butadiene levels were measured using personal samplers during the 6-h work shift and expressed as 6-h time-weighted average. These were supplemented with several grab samples. Three different work areas were identified: initial distillation and recovery from dimethyl formamide, polymerization, and recovery, with median air levels of 3.5, 1.0, and 1.1, respectively. The T cell cloning assay was performed from postshift blood samples. Unexposed subjects were age and gender matched and a brief questionnaire was administered. Tabular *hprt* mutant frequencies were presented grouped only by gender and exposed versus unexposed. Mean mutant frequencies were somewhat higher in females than males. Smoking (in males only) was not different in either group, but mutant frequency did significantly increase with age. Mean mutant frequencies, raw and adjusted for age, sex, cloning efficiency, and exposure status, were similar in exposed and nonexposed workers. Adjusted mean frequency for total exposed workers was 18.0 × 10^{-6} compared with 13.6 × 10^{-6} for nonexposed workers.

In a third study, Tates et al. (1996) used the T cell cloning assay on blood samples collected from workers in a butadiene plant in the Czech Republic. Workers were sampled in 1993 and 1994, but most of the blood samples from 1993 were lost to technical errors. A detailed analysis was conducted on the later group of 19 exposed and 19 nonexposed workers from other parts of the same plant. Personal samplers indicated a mean butadiene concentration of 1.76 ppm, with individual samples ranging from 0.012 ppm to 19.77 ppm. The geometric mean *hprt* mutant frequencies (adjusted for age, smoking, and cloning efficiency) were 7.10 × 10^{-6} for exposed and 10.59 × 10^{-6} for the controls. The range of mutant frequencies among

individuals was similar for both groups and individual mutant frequencies in the exposed group were not correlated with concentrations of butadiene detected in the personal samplers.

The results in both of the T cell cloning assay groups are clearly in conflict with the Ward et al. (1994, 1996b) findings both for exposed versus nonexposed and for smokers versus nonsmokers. A simple explanation would be that the increase in the autoradiographic assay was due to clones of mutants having arisen from earlier mutations. Even if true, the increase is clearly exposure related because 7 of the 8 exposed workers exhibited higher variant frequencies than the highest of the nonexposed controls. As indicated by Hayes et al. (1996), there are many differences between the two studies and currently no basis for rejecting either finding.

4.3. CYTOGENETIC EFFECTSCHUMAN

There have been four studies evaluating cytogenetic effects of exposed workers. Au et al. (1995) measured chromosome aberration frequencies in blood samples of 10 exposed workers and 10 matched controls from the same population used in the Ward et al. (1996b) study cited above. They reported measurable, but not significant ($p>0.1$), increases in chromosome aberrations and chromatid breaks. Also, cells were exposed to gamma-rays in G1 and aberrations were measured in the subsequent metaphase. With this indirect measure of DNA repair, chromatid breaks, deletions, and dicentrics were all significantly higher in cells from butadiene-exposed workers.

Sorsa et al. (1994) investigated chromosomal damage in blood lymphocytes sampled in 1993 from workers in the factories described by Tates et al. (1996) above. Chromosome aberrations, micronuclei, and sister chromatid exchange (SCE) frequencies were not elevated above samples from unexposed persons. They did note that smoking had a slight effect in micronuclei and SCE but not chromosome aberrations. Preliminary data measuring chromosome aberrations and micronuclei in blood samples from the 1994 group of workers was reported by Tates et al. (1996). The percentage of aberrant cells was significantly increased ($p<0.01$) in exposed subjects; however, the frequency of micronuclei in lymphocytes was similar in exposed and unexposed subjects. Evaluation of data for each subject would be required to determine the basis for the apparent discrepancy of the results between the two years.

The role of glutathione S-transferase (GST) genes GSTM1 and GSTT1 enzymes in the detoxification of butadiene metabolites has been evaluated by measuring the induction of SCE in cultured human lymphocytes. Uuskula et al. (1995) found that SCE induction in lymphocytes from GSTM1-null individuals was 31% higher than in lymphocytes from GSTM1-positive individuals when treated with 50 or 250 µM 1,2-epoxy-3-butene. The same group (Norppa et al., 1995) reported no difference in SCE induction among GSTM1 nulls and GSTM1-positive lymphocytes when treated in vitro with diepoxybutane; however, they observed a 60% increase

in SCE in lymphocytes from GSTT1-null individuals when treated with 2 or 5 µM diepoxybutane. Neither GSTM1 nor GSTT1 deficiency affected the induction of SCE by 250 or 500 µM of 3,4-epoxybutane-1,2-diol (Bernadini et al., 1996). In a separate study, Kelsey et al. (1995) found that GSTT1 deficiency significantly increased the frequency of SCE induced by diepoxybutane in lymphocyte cultures of workers exposed to butadiene. Hence while all three epoxides of butadiene metabolism are effective inducers of SCE in cultured human lymphocytes, there are differences in the role of at least two of the GST genes (GSTM1 and GSTT1) in the detoxification of the three metabolites.

4.4. CYTOGENETIC EFFECTSCRODENT

Most of the rodent in vivo cytogenetic studies on butadieneCespecially in somatic cellsChave been thoroughly treated in the reviews cited in the introduction of this chapter. In those studies, positive results were reported for all cytogenetic endpoints studied in mice and negative results were consistently reported in rats. Recent efforts have focused on cytogenetic effects in germ cells of butadiene as well as effects of the two primary epoxides of butadiene.

Butadiene induced dominant lethal effects in studies of male mice exposed by inhalation (Adler and Anderson,1994); the details are described in Chapter 4. That study was followed by an experiment measuring heritable translocations induced in exposed males (Adler et al., 1995). Males were exposed by inhalation to butadiene at 1,300 ppm for 5 days for 6 h/day. Offspring were tested for translocations by both litter size and cytogenetic analysis of meiotic and somatic cells. The translocation frequency from treated males was 2.7% compared with 0.05% for historical controls.

Xiao and Tates (1995) evaluated the cytogenetic effects of 1,2-epoxybutene (EB) and 1,2:3,4-diepoxybutane (DEB) in both somatic and germ cells of mice and rats. Male animals of both species received single i.p. injections of 40 or 80 mg/kg of EB. Animals were sacrificed at various time intervals after treatment and spleen and testes were processed for scoring of micronuclei. In splenocytes, EB was almost four times more effective in the mouse as in the rat. In mouse germ cells, the incidence of micronuclei was similar to controls on days 1 and 3 after exposure, but was significantly increased on day 14. In rats, EB was equally effective on days 1 to 3 (late spermatocytes) and day 20 (early spermatocytes) and the frequency of micronuclei at 80 mg/kg was slightly higher than that observed on day 14 in the mouse. For DEB, mice were injected with 15 or 30 mg/kg and rats received single i.p. injections of 20, 30, or 40 mg/kg. In a separate experiment, rats received 3 daily injections of 10 mg/kg. The response in splenocytes was similar in both mice and rats at 30 mg/kg. In mouse germ cells, DEB increased the frequency of micronuclei only on day 3 after treatment. Significant increases of micronuclei were observed in rat germ cells at all doses and all time periods. The results in somatic cells are

consistent with all other reports of greater sensitivity in mice than in rats. This difference is contradicted in germ cells with rats equally (or more) sensitive to micronuclei induction by both EB and DEB. The authors offered no explanation for this, stating that more research is needed to better understand the organ and species differences. It is noted that the strains of both species are different from those used in most other endpoint measurements. The mice were F_1 males of a (102 × C3H) cross of parent stocks from Adler's laboratory in Germany. The rats were Lewis rats supplied by Harlan CPB, the Netherlands.

Sjöblom and Lähdetie (1996) used an in vitro meiotic micronucleus assay to examine the effects of EB, DEB, and 1,2-dihydroxy-3,4-epoxybutane (diolEB) in seminiferous tubule sections of male Sprague-Dawley rats. Tissue sections were cultured for 4 days with EB at 100, 500, or 1,000 mol/L; DEB at 5, 10, or 20 µmol/L; or diolEB at 10, 50, or 100 µmol/L. The frequency of micronuclei was increased only by DEB and the increase was clearly dose-related. That EB was not effective is contrasted with the findings of Xiao and Tates (1995) above. The authors suggest that EB requires further metabolism by P450 enzymes, which they indicate does not occur in rat testes microsomes.

4.5. SUMMARY

The studies cited here along with the many earlier genotoxicity studies discussed in the cited reviews provide clear evidence that 1,3-butadiene is both mutagenic and clastogenic through its metabolism, primarily due to the mono- and diepoxide. While the difunctional DEB is clearly more effective than the monofunctional EB for most endpoints, it is not possible to ascribe the effects observed to one or the other when animals are exposed to butadiene. Where both have been studied, mice are more responsive than rats, except for the recent germ cell studies. Whether this exception is strain specific (among or between species) can only be answered with future work.

The role of GST is also clearly established for the genotoxic effects of butadiene in human lymphocytes. That the two glutathione S-transferases (GSTM1 and GSTT1) react differently with the three epoxide metabolites suggests that the relative concentrations of these metabolites will vary depending on the individual's genotype.